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Spectral Effects on the Growth Rate and Endocrine Histology of the Teleost, *Astyanax mexicanus*

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(Text-figures 1-4)

IT has been shown by Rasquin (1949) that prolonged maintenance of *Astyanax mexicanus* (Fillipi) in total darkness prevented normal functioning of the endocrine system. A depressed pituitary condition followed, together with thyroid hyperplasia, reduced gonads and retarded and deformed somatic growth. In the present experiment, *Astyanax* were maintained in daylight, under colored filters, in order to compare possible differential effects of specific regions of the visible spectrum with those produced by a lightless environment.

No previous studies directly concerned with wave length effects on the endocrines of fishes could be found in the literature, although the spectral influence on the growth of young salmon (Yung, 1881; Crawford, 1930) and on fish behavior (Kawamoto & Takeda, 1950) and color vision (Warner, 1931; Walls, 1942) has been described.

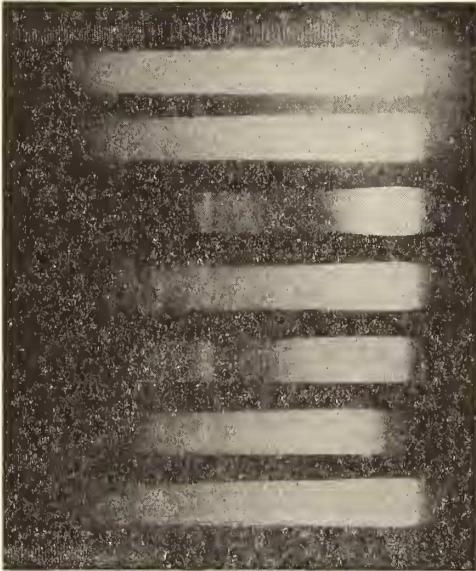
The experimental work reported here was carried out in the laboratory of the Department of Fishes and Aquatic Biology of the American Museum of Natural History. The author wishes to thank Dr. Charles M. Breder, Jr., and Priscilla Rasquin of this department for reading and criticizing the manuscript.

MATERIALS AND METHODS

Thirty fish of the same spawning, approximately two months old, and of about the same size, were equally distributed among five adjoining aquaria. These tanks, 16¼ by 8½ by 10 inches high, with glass sides and a slate bottom, contained only water. They stood on a stand six feet below the glass skylight of the aquarium room. All sides and the top cover of four of the tanks were covered respectively with red, yellow, green and blue cellophane sheets, with an opaque cardboard between the ends to eliminate influences between adjacent tanks. One tank, the control, was left without cellophane covering.

The yellow cellophane was manufactured by the Sylvania division of the American Viscose Corporation, and the red, green and blue, by the du Pont Cellophane Division. The extent to which the transmission of the filters varied was determined by spectrophotometric analysis, and is considered in the interpretation of the significance of the results. Daylight served as the only source of light. Aquarium room temperature during the nine-month experimental period (July through March) varied from 65 to 80 degrees F. All of the fish received a balanced daily diet of a standard dried food (Aronson's formula, 1949) which contained 12 percent. protein, 2 percent. fat and 32 percent. carbohydrate.

Monthly growth measurements were obtained as follows: the distance from the tip of the snout to the anal opening was recorded for length values, and the widest point in the body region for greatest depth values, measured at right angles to the axis of the fish. This measure of length was used because it could be more accurately determined on live fish than the more usual standard length, and was less susceptible to alteration than total length measurement. At the end of three months, and monthly thereafter for six months, one fish from each group was sacrificed for histological study of thyroid, pituitary and gonads. The fish were killed by immersion in Bouin's solution. Serial sections at 7 μ containing the pituitary and thyroid were stained with Masson's triple stain made up of ponceau-acid fuchsin and fast green. Serial sections through the body region containing gonads were cut at 10 μ and were stained with Harris's hematoxylin and eosin. The epithelial cell heights of the thyroid follicles were measured by a modification of Rawson and Starr's method (1938), and the mean cell heights were compared for statistical significance using the standard formula for the derivation of the value of $d/\sigma d$.



TEXT-FIG. 1. Spectrograms of colored filters obtained with a quartz spectrograph and a carbon arc lamp. Numbers 1, 2 and 7 represent the transmission of the carbon arc lamp alone; 3, 4, 5 and 6 represent respectively the transmission of the red, yellow, green and blue filters.

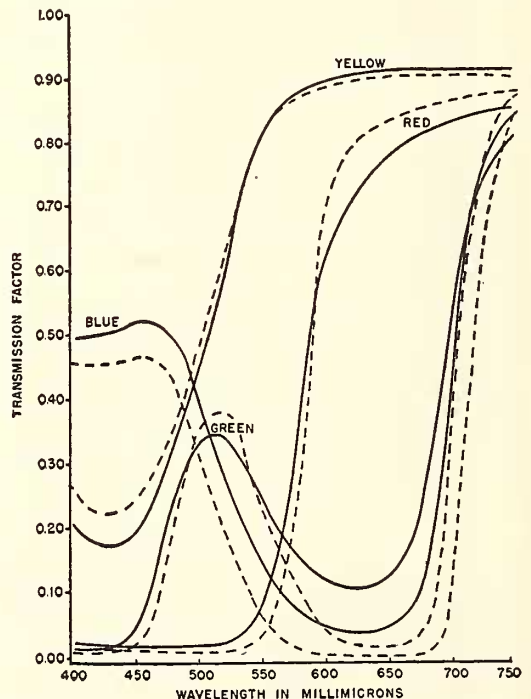
The luminous transmission of the colored filters, in foot candles, at the bottom of each tank, when empty, under average daylight conditions, was determined with a Norwood Director exposure meter, Model B, number 261, American Bolex Company. These values were respectively: control, 510; red, 135; yellow, 490; green, 100; blue, 110.

The spectral transmission by the colored filters was determined by means of a quartz spectrograph, with a carbon arc lamp as a light source. Although the carbon arc spectrum extends to include some of the ultraviolet, the transmission of these shorter wave lengths by the colored filters was of no concern because these wave lengths were blocked by the window glass skylight as well as by the sides and top of the aquaria. The spectrograms in Text-fig. 1 indicate that all of the filters except the red transmitted some of both the longer and the shorter wave lengths of visible light.

A more detailed analysis of the wave lengths transmitted by the colored filters was obtained from spectrophotometric tests on samples of the cellophane both before and after use, since it was possible that considerable fading could have occurred. These tests were carried out at the Electrical Testing Laboratories, New York City, with a Hardy Recording Spectrophotometer. The two series of transmittance curves are in-

corporated into Text-fig. 2. It is seen that the differences in transmission between the used and unused samples were small at most wave lengths. The yellow filter transmitted the most light, followed by the red filter, but the blue and green filters transmitted considerably reduced amounts of light. At the shorter wave lengths of 400 to 550 millimicrons, which included violet, blue and most of the green regions, transmission by the red filter was low enough to be considered insignificant. Although the green and blue filters transmitted only small amounts of the yellow and orange wave lengths, they transmitted about 80 percent. of the far red region of the spectrum.

From the energy distribution in the spectrum of a standard illuminant (C), which is an approximation of average daylight established by the International Commission for Illumination (Judd, 1933), it was seen that the energy content at 450-550 millimicrons exceeded that of all other spectral regions. This is the region blocked by the red filter, so that although more light was transmitted, radiant energy under this filter was probably no greater than under the blue and



TEXT-FIG. 2. Transmittance curves of the cellophane filters obtained from spectrophotometric tests with a Hardy spectrophotometer. The broken lines represent the transmission by the unused filters at the start of the experiment, and the solid lines represent the transmission by the used filters at the end of the experiment.

TABLE 1. MONTHLY MEAN LENGTH AND DEPTH IN MILLIMETERS OF FISH UNDER COLORED FILTERS

Filter	Number of Fish	Age in Months	Mean Length	Standard Error	Mean Depth	Standard Error
Control	6	2	20.7	.653	8.3	.304
	6	3	27.0	.527	9.5	.392
	6	4	30.8	.843	10.5	.391
	6	5	33.2	.843	10.8	.487
	5	6	34.4	.828	11.4	.455
	4	7	36.0	1.060	12.2	.416
	3	8	36.3	1.502	10.2	.606
	2	9	31.0*	11.0	...
	1	10	35.0	10.0	...
Red	6	2	20.7	.561	8.5	.312
	6	3	24.3	1.326	9.2	.367
	6	4	30.0	1.027	10.8	.548
	6	5	31.4	1.194	10.7	.451
	5	6	33.2	1.244	10.6	.455
	4	7	34.0	1.658	11.2	.829
	3	8	34.6	2.231	10.1	1.097
	2	9	33.5	9.5
	1	9½	30.0	10.0
Yellow	6	2	19.0	.472	7.3	.304
	6	3	25.0	1.312	8.7	.509
	6	4	30.7	1.018	10.5	.565
	6	5	31.5	.874	10.3	.561
	5	6	32.8	.912	10.0	.565
	4	7	34.0	.612	10.7	.650
	3	8	31.3	1.658	8.6	.545
	1	8½	35.0	9.0	...
	1	9	32.0	9.0	...
Green	6	2	20.5	.514	8.0	.236
	6	3	25.3	.765	9.0	.333
	5	4	28.4	1.252	10.2	.438
	5	5	31.6	.456	10.2	.334
	4	6	33.0	.707	10.5	.500
	3	7	34.0	.943	11.3	.236
	2	8	34.5	9.5	...
	1	9	36.0	11.0	...
Blue	6	2	20.7	.452	8.3	.193
	6	3	26.7	1.240	9.7	.304
	6	4	30.8	1.906	10.0	.707
	6	5	31.5	1.594	10.5	.612
	5	6	32.8	1.682	10.6	.799
	4	7	34.5	1.479	12.0	.935
	3	8	32.3	2.054	9.3	.981
	2	9	35.5	9.5	...
	1	9½	30.0	10.0	...

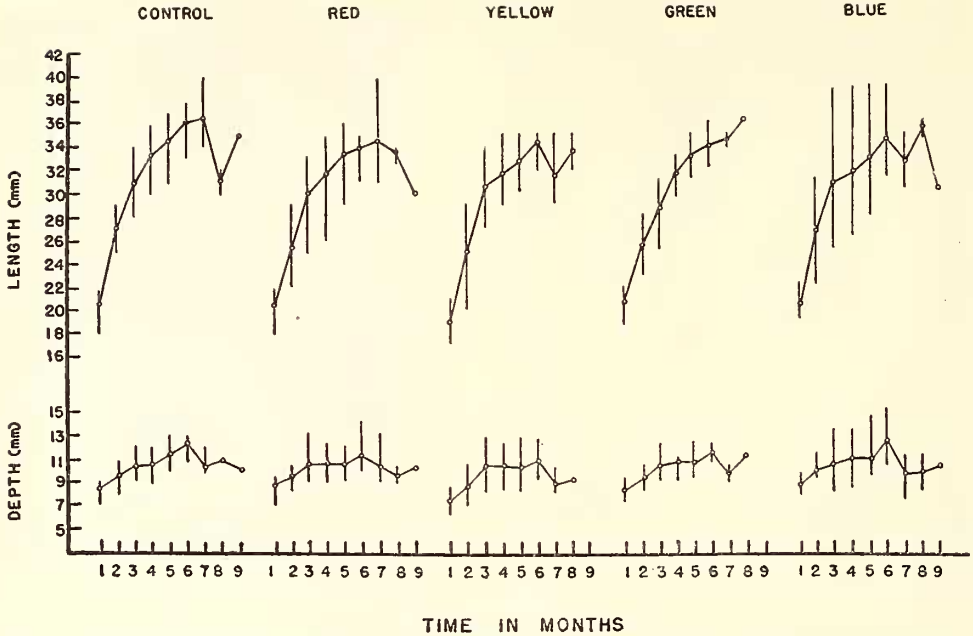
* Standard errors not calculated when less than three fish left in group.

green filters, whereas radiant energy under the yellow filter was considerably greater, although still inferior to the controls.

RESULTS

General behavior.—No behavior derange-

ments could be attributed to the colored filters. As far as could be determined, feeding was normal and regular in all of the groups. *Astyanax mexicanus* is known to exhibit frequent "nervous" behavior patterns. In the course of one or several days these fish sometimes alternate



TEXT-FIG. 3. Monthly mean lengths and depths of the fish are indicated by small circles. Monthly extreme values of length and depth are indicated by vertical lines.

from a relatively quiescent state to an extremely active hostile condition, which occasionally results in a group annihilation (Breder, 1943).

Survival.—There were no differences in the ability to survive under the colored filters. During the course of the experiment one fish from each group except the control died from unknown causes. It appeared, however, that death was not caused by the experimental environments.

Growth.—The filtered light did not inhibit the body growth of the fishes, as seen from the data in Table 1, where the monthly mean lengths and depths and their standard errors are listed. The growth curves (Text-fig. 3), prepared from the mean lengths and depths plotted against time, have similar slopes, except in the last few months of the experiment. The fluctuations seen at this time were caused by a reduced number of fish as they were killed for histological study. Comparisons of the monthly size ranges of the fish, represented by the vertical lines in Text-fig. 3, indicate that the most extreme size differences were shown by the fish under the red and blue filters.

The greatest percentage of growth in mean length and depth at the end of five months occurred in the group under the yellow filter, and in the controls, whereas under the red filter this value was smallest. From the percentage growth

at monthly intervals it was found that growth during the first month under the red filter was small, but by the end of the second month increased to the greatest value found in any of the other groups (Table 2). This probably reflects a slower adjustment to the red filter. Statistical examination of the data in this table indicates that these growth differences were not statistically significant.

Thyroid.—In the thyroid glands of *Astyanax* under one year old, maintained under normal laboratory conditions, the follicular epithelium varies from squamous to low cuboidal, and occasionally high cuboidal. The location of the follicles, which are diffusely scattered as in most other teleosts, and their normal histological structure, were described by Rasquin (1949).

No extreme deviations in thyroid histology were produced by the colored filters. Monthly variations in the height (Table 3) and activity of the follicular epithelial cells and differences in the amount and staining reaction of the colloid were seen in all of the groups. The thyroid hyperplasia observed by Rasquin (1949) in *Astyanax* maintained in complete darkness was not produced in these fish.

Small differences in thyroid activity revealed by careful analysis of the data are of sufficient interest to be further examined. It can be seen from the graphic representation of the mean

TABLE 2. PERCENTAGE INCREASE IN MEAN LENGTH AND DEPTH AT MONTHLY INTERVALS
AND AFTER A FIVE-MONTH INTERVAL

Months	Number of Fish		Percentage Increase Under Colored Filters				
			Control	Red	Yellow	Green	Blue
Mean Length							
1	-	6	41.2	27.1	40.0	35.6	43.5
2	-	6	24.8	42.9	38.0	23.0*	29.7
3	-	6	15.7	10.5	5.3	23.7	5.1
4	-	5	7.8	13.5	8.7	10.4	9.4
5	-	4	10.5	6.0	8.0	7.4	12.3
1-5		—	73.9	64.3	78.9	65.9	67.5
Mean Depth							
1	-	6	30.8	24.1	38.9	30.3	37.8
2	-	6	25.6	55.2	46.2	36.4*	8.1
3	-	6	7.7	decr.	decr.	00.0	13.5
4	-	5	15.4	decr.	decr.	9.1	2.7
5	-	4	20.5	20.7	17.9	24.2	37.8
1-5		—	47.0	31.8	46.6	41.3	44.6

* Average of five fish.

thyroid epithelial cell heights (Text-fig. 4) that the control group showed the smallest variations in height, whereas all of the colored filters produced more atypical curves when compared with the controls, especially the blue and yellow filters. The values for the difference between the extremes of the mean thyroid epithelial cell heights were respectively: blue, 1.01; yellow, .70; red, .59; green, .56; control, .29 (all in microns).

Over 50 percent. of these variations in the mean thyroid cell height associated with the colored filters were found to be statistically significant, as seen in the lower part of Table 3. The mean cell heights were significantly decreased by the yellow filter, increased by the green and red filters, and both decreased and increased, in different fishes, by the blue filter.

Other changes in thyroid histology accompanied the variations in cell height. When cell height was high, the follicles were small, the epithelial cells contained vacuolated cytoplasm, and the stored colloid which had decreased in amount and changed in staining reaction, contained chromophobic vacuoles. When cell height was low, the follicles were enlarged and were filled with deeply staining dense colloid.

Pituitary.—The examination of serial sections revealed no changes in the histology of this gland that could be referred to the colored filters. The relative proportions and the size, distribution,

cytoplasmic granulation and staining reactions of the acidophiles, basophiles and chromophobes of the transitional lobe—normally associated with cyclic changes in secretory activity—corresponded to the description given by Rasquin (1949) for the normal six- to twelve-month-old *Astyanax* pituitary. Vacuolated basophiles, normally found in this lobe, were more numerous in the five- to eight-month-old fish grown under the blue, green and yellow filters, than in the controls of the same age, whereas in all of the nine-month-old fish many vacuolated basophiles were present.

Ovary.—Fully mature yolk-filled ova were found in nine of the thirteen ovaries examined. Under each colored filter, however, there was one fish with only a few mature eggs. The data were insufficient to evaluate this finding because under similar normal conditions it is known that some fish mature earlier and develop faster than others. Some resorbing eggs, probably caused by a failure to ovulate, were found in all ovaries. This resorption process was best observed in the extremely large, fully mature ovaries from the seven-month-old control fish, and the six-month-old fish under the yellow filter. In the middle and posterior regions of these ovaries, all of the mature eggs appeared to be breaking down. They had become confluent into one large mass which contained eosinophilic yolk, fat-like vacuoles, reticular-like tissue, cho-

TABLE 3. INDIVIDUAL AND GROUP MEAN THYROID EPITHELIAL CELL HEIGHTS IN MICRONS OF FISHES UNDER COLORED FILTERS

Filter	Age in Months	Sex	Size in mm. Length by Depth	Mean Cell Height	Standard Error	Group Mean Cell Height	Standard Error
Control	5	M	34x11	2.585	.0368	2.55	.0444
	6	F	34x11	2.725	.0356		
	7	F	38x13	2.510	.0399		
	8	F	40x12	2.500	.0400		
	9	F	32x11	2.435	.0358		
Red	5	F	32x10	2.600	.0339	2.65	.2761
	6	M	34x11	3.010	.0464		
	7	M	35x10	2.663	.0429		
	8	F	40x13	2.420	.0417		
	9	M	34x9	2.575	.0378		
Yellow	5	F	35x12	2.670	.0443	2.36	.3623
	6	F	33x11	2.625	.0335		
	7	F	35x12	2.300	.0367		
	8	M	35x10	1.970	.0468		
	9	M	32x9	2.280	.0285		
Green	5	M	31x9	2.800	.0452	2.62	.3052
	6	F	31x11	2.935	.0375		
	7	F	36x12	2.380	.0395		
	8	M	34x9	2.425	.0389		
	9	M	36x11	2.545	.0368		
Blue	5	M	30x10	3.255	.0631	2.70	.1536
	6	F	34x11	2.655	.0379		
	7	M	39x15	2.250	.0391		
	8	M	35x11	2.470	.0528		
	9	F	35x11	2.855	.0334		

Comparisons made for statistical significance of individual mean thyroid cell heights

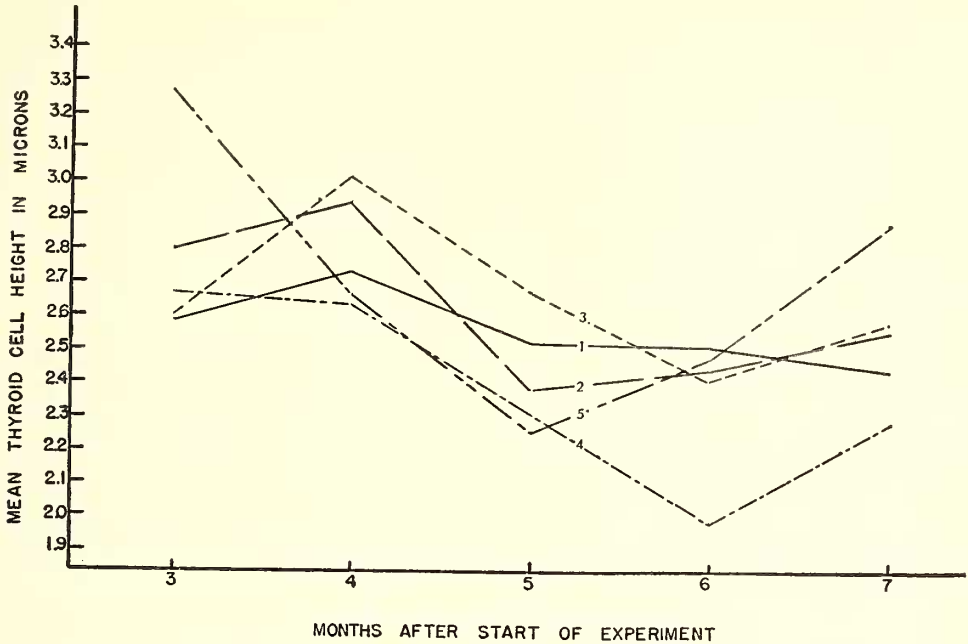
Fishes Compared	Significance
5 mos. control with 5 mos. green	3.6
5 mos. control with 5 mos. blue	9.2
6 mos. control with 6 mos. red	4.9
6 mos. control with 6 mos. green	4.1
7 mos. control with 7 mos. red	2.5 prob. sig.
7 mos. control with 7 mos. yellow	3.8
7 mos. control with 7 mos. blue	4.6
8 mos. control with 8 mos. yellow	8.6
9 mos. control with 9 mos. red	2.7
9 mos. control with 9 mos. yellow	3.5
9 mos. control with 9 mos. blue	8.5

ronic membrane remains and hyperplastic follicular epithelium.

In three of the four fish with ovaries that contained few mature eggs, many ova were resorbing prematurely. It is of interest that these fish were seven to nine months old (from the groups under the blue, green and yellow filters). In the five-month-old fish from the group under the

red filter that was not yet fully mature, resorbing eggs were not found.

Testis.—The testis is organized into lobules which contain cysts of spermatogenic cells. As the cells mature they are shed into the lobular cavities where they are stored prior to passage into the sperm duct. Rasquin & Hafter (1951) described the normal structure of the testicular



TEXT-FIG. 4. Mean thyroid epithelial cell heights, from three to seven months after the start of the experiment, for all of the fish. Numbers 1, 2, 3, 4 and 5 represent respectively the controls and the groups under the green, red, yellow and blue filters.

lobules and Rasquin (1951) demonstrated the presence of osmicated granules in the sperm duct epithelium which are indicative of a probable secretory function.

Only one of the twelve males examined was from the control group. This made difficult the determination of specific effects of the filtered light. It was observed, however, from comparisons of serial sections through corresponding regions, that all of the testes contained mature sperm in the lumina of the lobules and in the sperm duct.

In five of the seven- to nine-month-old fish from the experimental groups (two from yellow, one each from the red, green and blue groups) the testes contained very little spermatogenic tissue. This was also observed in some of a series of control males of approximately the same age, examined for another experiment, which indicates that this lack was probably not caused by the filtered light.

DISCUSSION

The reduction of luminous intensity and radiant energy by the red, green and blue filters, compared with the controls, could account for the decreased total growth and increased thyroid activity observed in these groups. Similar but vastly greater changes were found when *Asty-*

anax were maintained in total darkness (Rasquin, 1949). Yung (1881) reported a spectral effect on the growth rate of salmon and tadpoles, but he used filters that differed in the transmission of radiant energy. Rugh (1935) analyzed the properties of the filters used by Yung and found that the transmission of the most radiant energy resulted in the fastest growth. Rugh also found, in his own experiments, that when radiant energy was equalized, differences in wave length and luminous intensity produced no differential effects on the growth rate of tadpoles. This relationship between radiant energy and growth rate was also found in these experiments on *Astyanax*. The red filter transmitted more light than the blue and green filters, but about the same amount of radiant energy. The percentage increase in length was approximately the same under all three filters. The absence of most of the shorter wave lengths under the red filter may also have contributed to the small length increase. The presence of both the shorter and longer wave lengths of visible light were found to be essential for the normal growth of young chicks (Sheard & Higgins, 1928; Sheard, Higgins & Foster, 1930). Constant housing of these chicks behind an amber glass filter that transmitted only the longer visible wave lengths of sunlight retarded growth and development.

Crawford (1930) noted that blue light resulted in the slowest rate of growth of young salmon, and also the heaviest mortality; red light produced growth effects similar to those produced by total darkness; and green and yellow light were optimal for growth. He did not publish any data, however, on the properties of the filters he used. Red and blue light were found to accelerate the growth of young rats (Ludwig & von Ries, 1931), but this was not confirmed by Allardyce, et al. (1942), who found no consistent differences in growth rate following maintenance under colored, colorless and black filters.

The variability in the growth of young fishes often encountered under normal conditions may be partially responsible for the small differences observed in the groups under the colored filters. Consideration should also be given to the difference in sex distribution in the different groups, since the fish were sexually immature at the start of the experiment. In *Astyanax*, females usually attain a larger size than males, so that the predominance of females in the control and yellow groups may therefore, in part, explain the greater total growth that occurred in these groups.

Modlinger (1941) found that red light and darkness increased the epithelial cell height and decreased the iodine content of the colloid of the dove thyroid, and these changes coincided with those that took place during the annual cycle. Pighini (1941) also reported that monochromatic red, yellow, green and blue light all produced increased thyroid activity in guinea pigs. The effects were not wave-length specific, and may have been caused by the decreased light and radiant energy transmitted by the colored lights. In *Astyanax*, the yellow filter significantly decreased thyroid cell height, and this appeared to be a wave-length effect, since the light and radiant energy transmitted did not differ substantially from the control. The blue filter significantly increased thyroid cell height in two fish, but significantly decreased cell height in another fish. The increased cell height was explained by a decreased transmission of light and radiant energy, but it is impossible to explain the opposite effect without additional data. It is possible that these results, although statistically significant, are not biologically significant because of the nature of the material.

Other studies also indicated that decreased light or darkness results in increased thyroid activity (Bergfeld, 1931, in rats; Puntriano & Meites, 1951, in mice; and Rasquin, 1949, in the teleost, *Ameiurus nebulosus*), although Mayerson & Branch, 1934; Mayerson, 1935; and

Kenyon, 1935, in rats; and Stein & Carpenter, 1943, in the salamander, *Triturus viridescens*, did not confirm this response.

That all of the colored filters altered the thyroid activity of *Astyanax* indicates the role of this gland in the general response to a changed environment. An altered metabolic rate may have been involved in this response, although it is not definitely known that the teleost thyroid gland is a regulator of metabolism (Etkin, Root & Mofshin, 1940, and Matthews & Smith, 1947). Some indication of the thyroid's metabolic function is seen by the reports that total darkness increased both the oxygen consumption (Schlagel & Breder, 1947) and the thyroid activity (Rasquin, 1949) of *Astyanax*.

Although no prominent changes in the pituitary of *Astyanax* were produced by the colored filters, Pighini (1941) found that red, yellow, green and blue light all produced the early appearance of acidophiles in the anterior lobes of young guinea pigs. This was attributed more to the lower luminous intensity and radiant energy transmitted by the colored lights than to specific wave-length effects. In addition, darkness reduced the relative number and activity of pituitary basophiles in *Astyanax* (Rasquin, 1949) and in frogs (Florentin & Stutinski, 1936; Stutinski, 1936; and Woitkewitsch, 1944).

The many resorbing ova found in all of the ovaries of *Astyanax* did not appear to be effects of specific wave lengths. The breakdown of mature ova, in the absence of spawning, and their subsequent resorption, has been described as a normal occurrence in other teleost ovaries (Brock, 1878; Barfurth, 1886; Cunningham, 1897; and Wallace, 1904). These degenerating ova undergo fatty changes, accompanied by the immigration of much cell debris into the substance of the egg. It is still uncertain whether this cell debris comes from a growth and proliferation of the follicular epithelium (as in trout ova, Barfurth; and in *Zoarcetes viviparus*, Wallace), from a proliferation of the cells from the connective tissue in the wall of the follicle (as in *Trigla hirunda*, Cunningham), or from both of these locations (as in the bitterling, *Rhodeus amarus*, Bretschneider & de Wit, 1941).

It has been demonstrated by the present work that despite all of the spectral, light intensity and radiant energy reductions provided by the colored filters, the fish were able to maintain the essential integrity of their endocrine systems. Evidently, for this stability, the presence of some visible radiation as such is of greater significance than the exact spectral quality of that radiation, for which there are many corroborating studies

in the literature. There is need for further experiments which will utilize filters of more refined properties and which will operate at intensities between those used in the present study and the zero intensity used by Rasquin (1949). The minimal light intensity that will support normal endocrine structure and function, as well as the possible importance of specific wave lengths at such a lowered intensity of light, are problems yet to be studied.

SUMMARY AND CONCLUSIONS

1. Two-month-old *Astyanax mexicanus* were grown under daylight filtered by red, yellow, green and blue cellophane sheets, for three to eight months, after which the histology of the thyroid, pituitary and gonads was compared with that of controls maintained without any filter. The cellophane sheets were analyzed for the transmission of the spectrum and for luminous intensity.

2. At the end of five months, the fish under the yellow filter showed about the same percentage of increase of growth in body length and depth as the controls. A smaller percentage of increase was attained under the red, green and blue filters, because of the decreased light transmitted, but these lower figures were not significantly different from the controls.

3. Thyroid activity in some of the fish under the red, green and blue filters was significantly increased over the controls, and was probably produced by the decreased transmission of these filters. Some spectral influence of the yellow filter was presumed to be responsible for the significantly decreased thyroid activity observed in some of these fish. Additional data are needed to explain the significantly decreased thyroid cell height in one fish grown under the blue filter. The biological significance, if any, of these alterations in thyroid activity is yet to be ascertained.

4. The structure of the transitional lobe of the pituitary and the testes showed no significant changes induced by the colored filters.

5. Some ovaries that were not fully mature were found in fish raised under the colored filters, with prematurely resorbing ova, especially under the yellow, green and blue filters. The significance of this item is unknown.

6. It was demonstrated that despite all of the spectral, light intensity and radiant energy reductions provided by the colored filters, the fish were able to maintain the essential integrity of their endocrine systems.

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